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Supplementary Information for

Mitochondrial apoptotic priming is a key determinant of cell fate upon p53 restoration

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Supplementary Figures 1 to 6

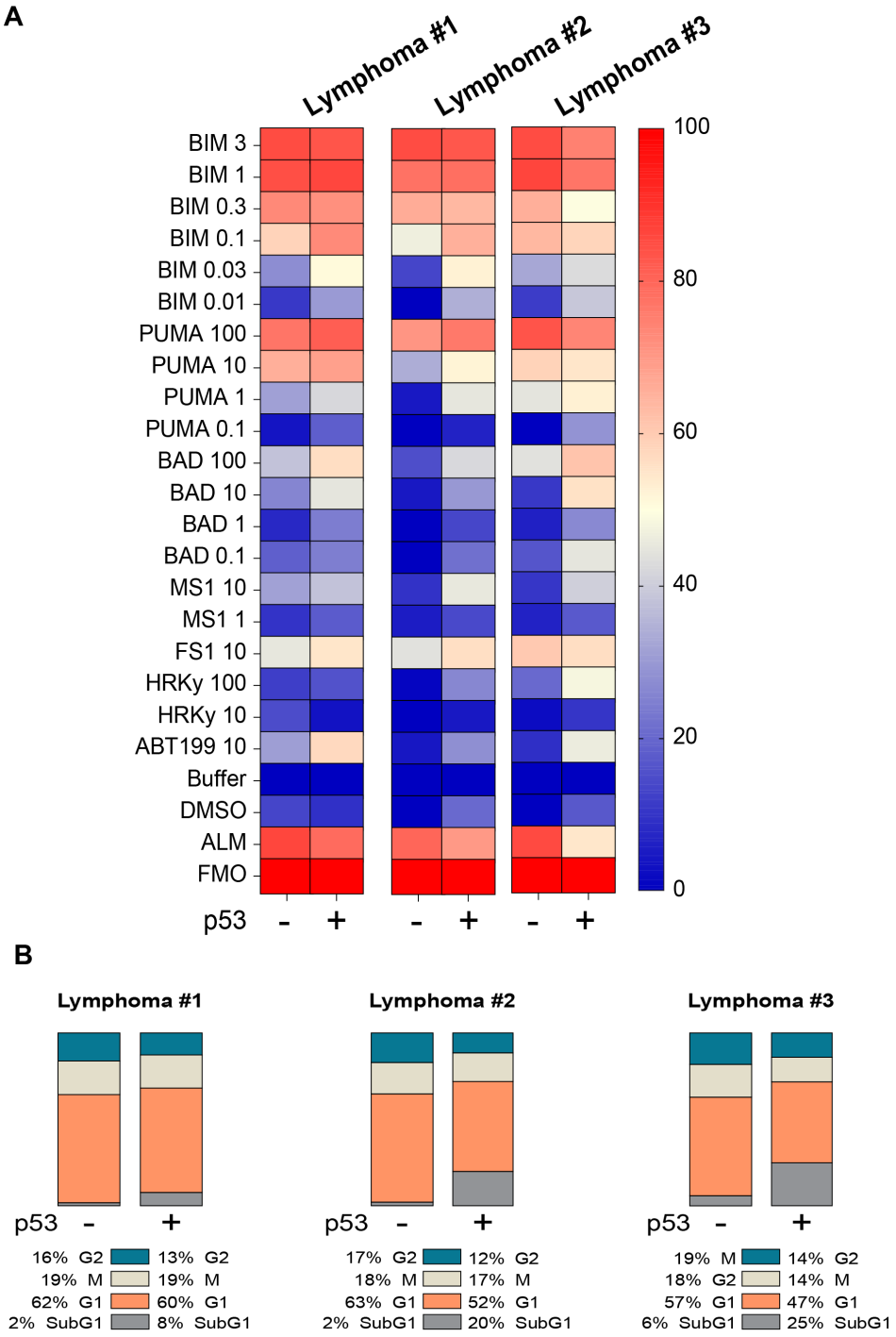

Figure S1: Restoration of p53 increases mitochondrial priming in lymphoma cells. A Hoechst 33342 stained lymphoma cells unrestored (-) or p53 restored (+ 4OHT) show increased priming at 24 hours to a variety of peptides. Only cells with intact nuclei (G1-M-G2) were analyzed by flow cytometry using iBH3 profiling (see Methods). **B** DNA content of lymphoma cells at 24 hours post p53 restoration.

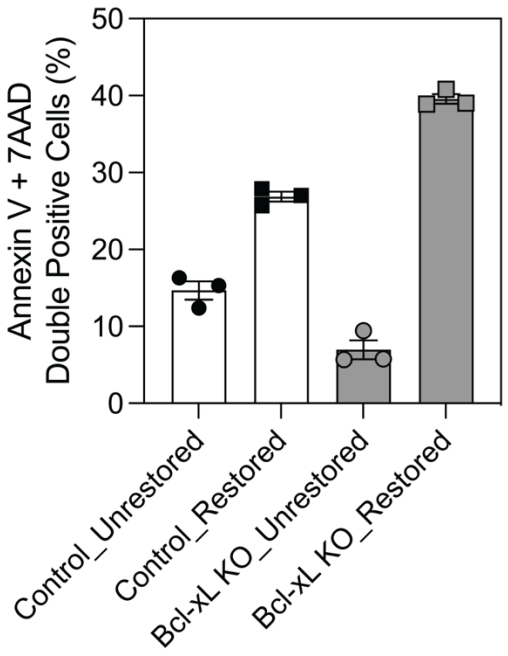

Figure S2: *Bcl-xL* deletion switches the fate of p53-restored lung adenocarcinoma cells from cell cycle arrest to apoptosis. Percentage of Annexin V-7AAD double positive control or *Bcl-xL* knockout cells 72hrs after p53 restoration. Data represents the mean ± S.E.M, n=3 or more. Statistics were calculated with two-sided Student's t-test: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

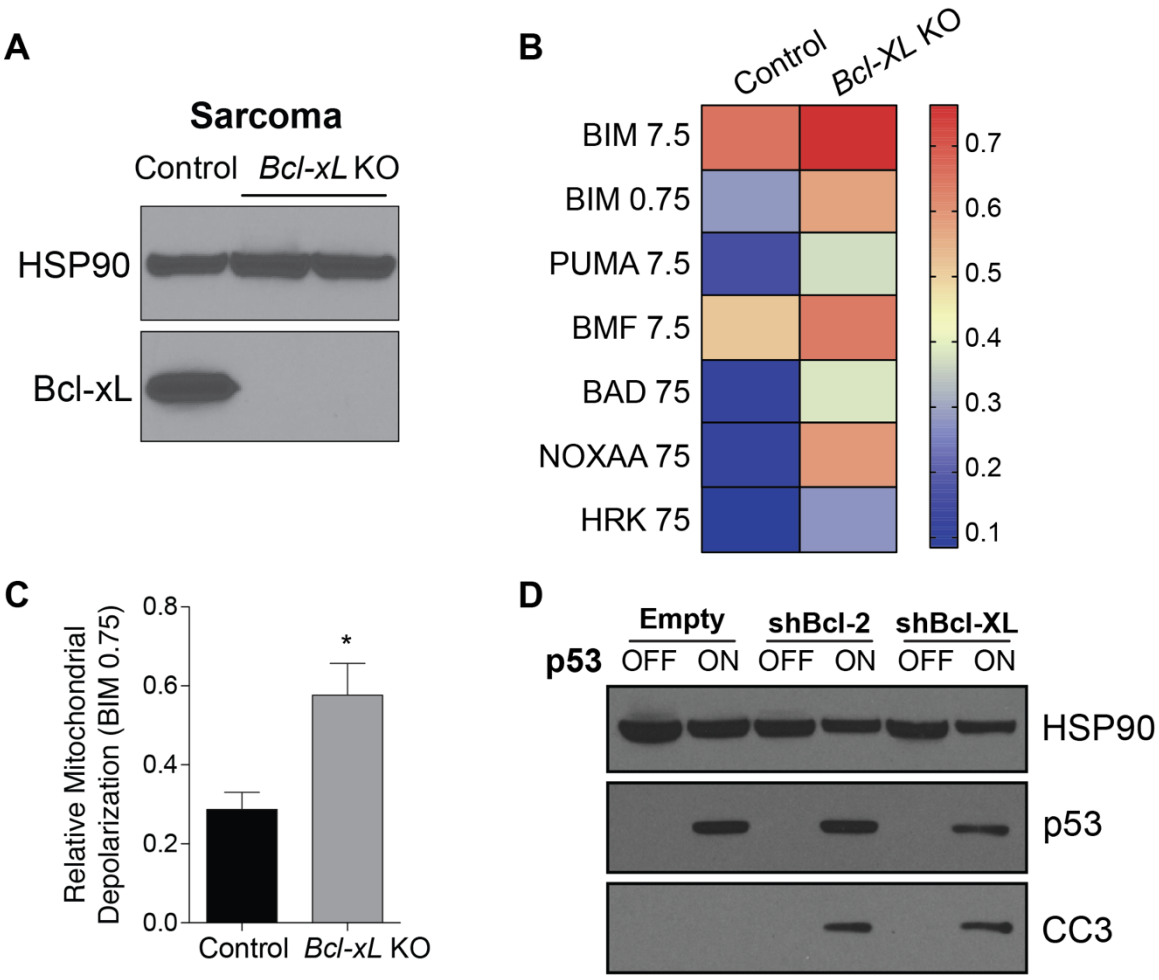

Figure S3: Genetic priming of sarcoma cell lines is sufficient to switch cell fate of p53-restored cells from cell cycle arrest to apoptosis. A CRISPR-mediated deletion of *Bcl-xL* in sarcoma cell lines. **B** BH3 profile of a representative *Bcl-xL* knockout sarcoma cell line showing that *Bcl-xL* deletion increases mitochondrial apoptotic priming. **C** Priming measured by BIM peptide is significantly increased in *Bcl-xL* knockout sarcoma cell lines. **D** *Bcl-2* or *Bcl-xL* knockdown is sufficient to switch cell fate of p53-restored sarcoma cells from cell cycle arrest to apoptosis as gauged by increased levels of CC3. Data shown is from cells harvested 72hrs after p53 restoration. Data in (C) represent the mean ± S.E.M, n=3 or more. Statistics were calculated with two-sided Student's t-test: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

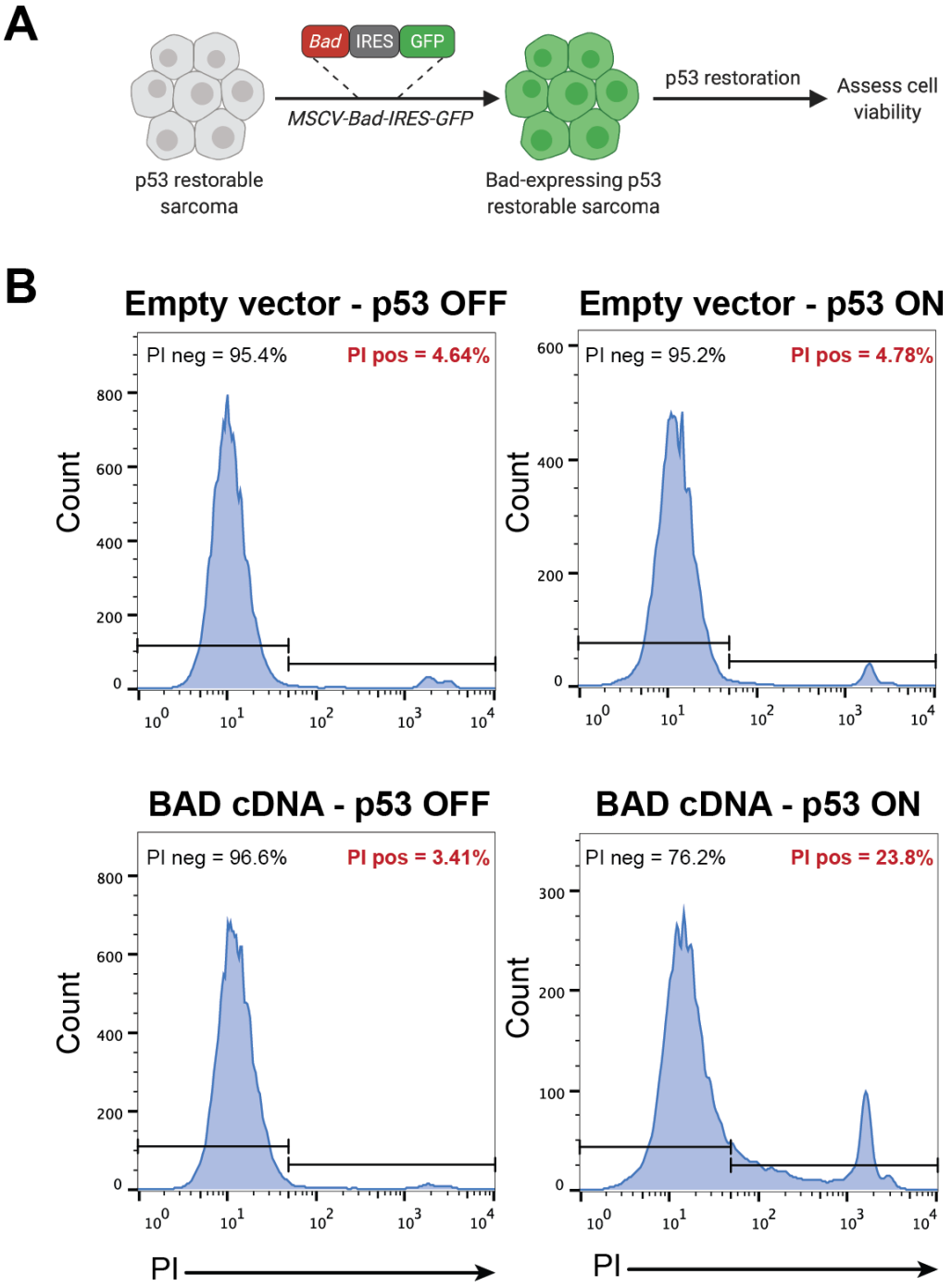

Figure S4: Increased mitochondrial priming by forced expression of BAD is sufficient to switch the fate of sarcoma cell lines to apoptosis *in vitro*. **A** Generation of *Bad*-overexpressing sarcoma cell lines using MIG-Bad (or MIG-Empty as control). Diagram created with BioRender.com. **B** Viability of MIG-Empty of MIG-Bad sarcoma cell lines at baseline (left panels) or at 72hrs after p53 restoration (right panels) measure using propidium iodide (PI) exclusion.

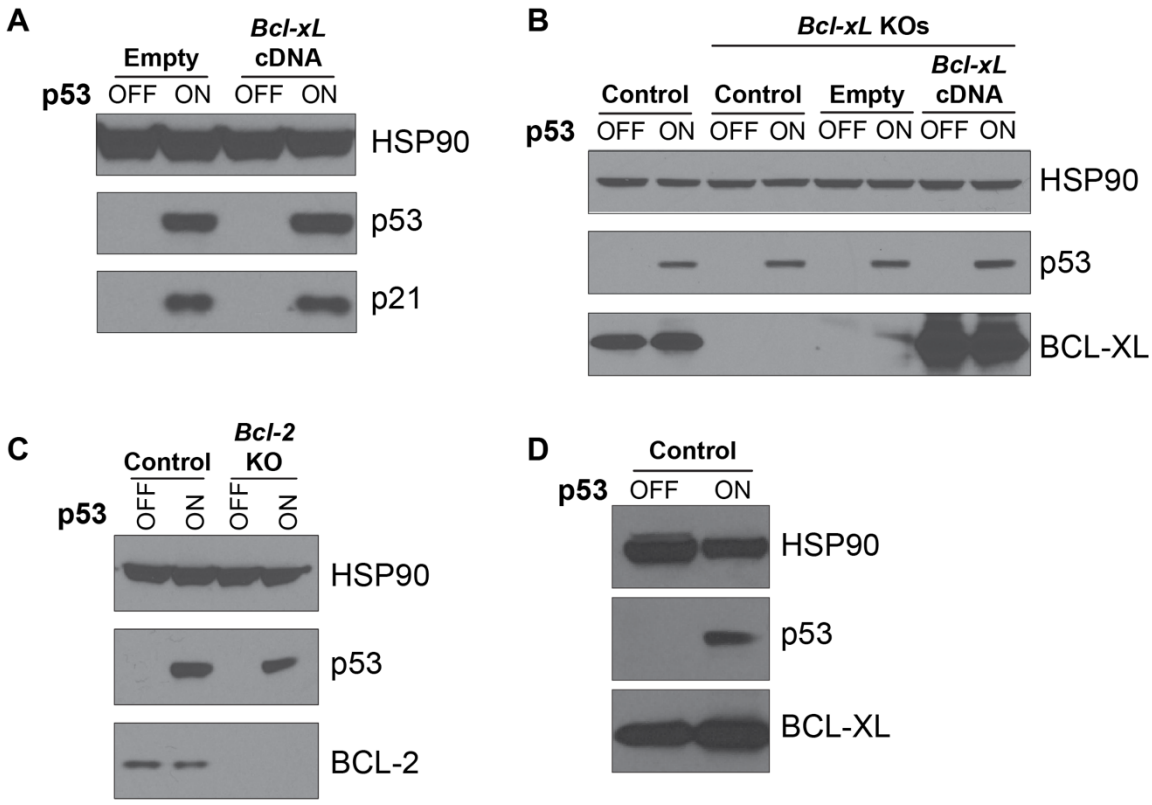

Figure S5: Genetic manipulation of *Bcl2* or *Bcl-XL* does not impact p53 levels or signaling post-restoration. A Western blot of lysates obtained from lung adenocarcinoma cells transduced with either MIG-Empty or MIG-Bcl-xL at baseline or after p53 restoration for 72 hours. **B** Western blot of lysates obtained from parental or *Bcl-XL* knockout lung adenocarcinoma cells transduced with either MIG-Empty or MIG-Bcl-xL at baseline or after p53 restoration for 72 hours. **C** Western blot of lysates obtained from parental or *Bcl-2* knockout lung adenocarcinoma at baseline or after p53 restoration for 72 hours. **D** Western blot of lysates obtained from sarcoma cells at baseline or after p53 restoration for 72 hours.

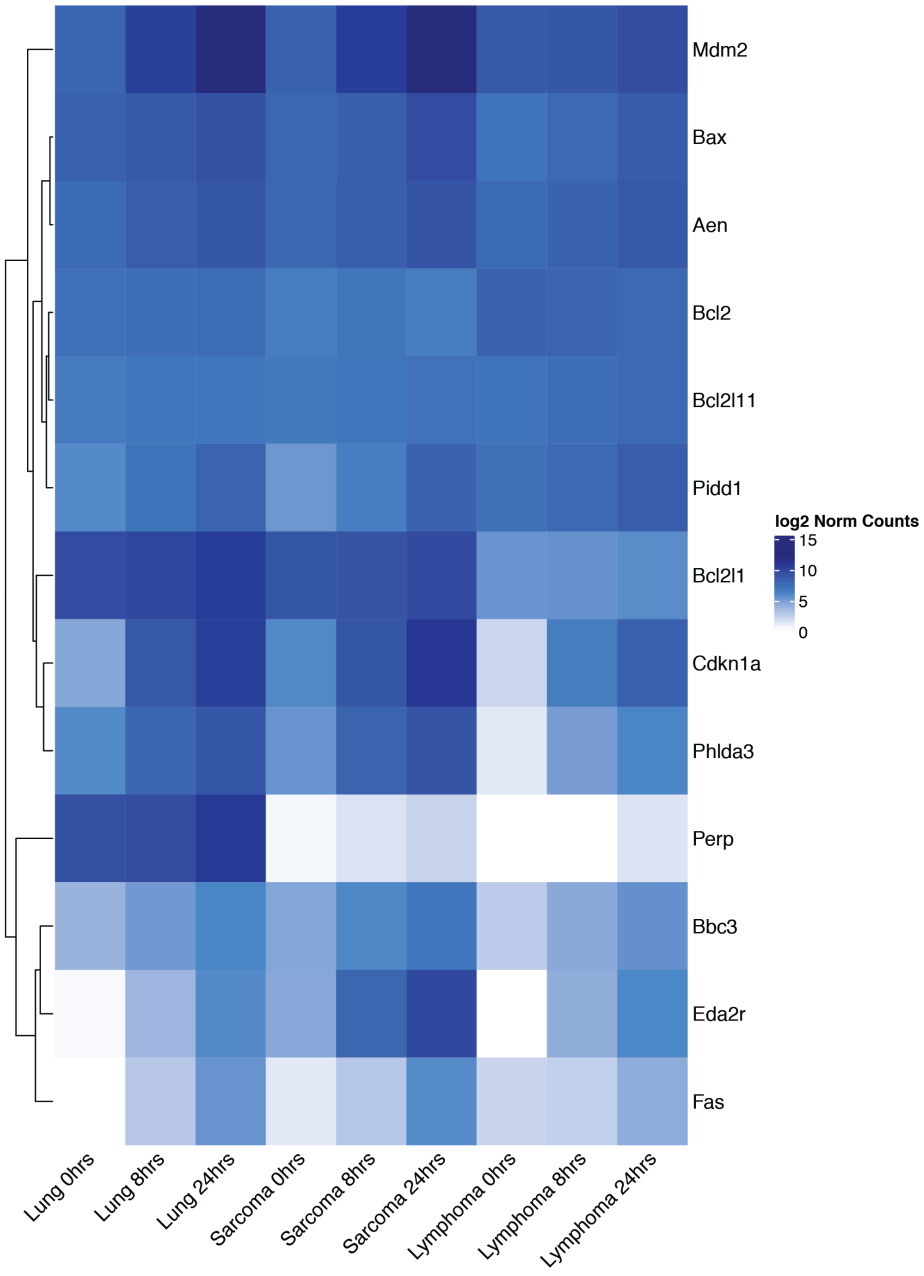

Figure S6: RNA sequencing analysis of core p53 target genes and selected Bcl2 family members. Heatmap visualization of log2 normalized count values from RNA sequencing data obtained from lymphoma, sarcoma, and lung adenocarcinoma cells undergoing p53 restoration (see accompanying manuscript from Tesfaye et al.).